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Rejection — More Than the Eye Can See

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In comparing current clinical outcomes in renal transplantation with those of 30 years ago,[1] graft failure from immunologic factors and death from opportunistic infection in the first year after transplantation are no longer common clinical outcomes. The therapeutic regimens used today to prevent and treat rejection or infection among renal-transplant recipients bear only a small resemblance to those used 30 years ago.

In contrast, the diagnostic strategies used to detect rejection and distinguish it from other causes of renal dysfunction have not budged during the past two decades. A rise in the level of serum creatinine suggests allograft dysfunction, but the reasons can be elusive. Nephrotoxicity from immunosuppressive agents may cause acute and chronic allograft dysfunction, and accordingly, clinicians attempt to maintain drug levels in the therapeutic range. To aid in the resolution of the difficult differential diagnosis of allograft dysfunction, ultrasonography and renal biopsy are often performed.

This reactive diagnostic approach is often too late, and there are limitations because, first, drug levels do not test how therapy is affecting the recipient's immune response, and second, biopsies lack sensitive histologic patterns for the diagnosis of drug-induced nephrotoxicity and early rejection. Furthermore, detecting adverse host antigraft immunity before there is evidence of graft dysfunction has not been feasible. Since the diagnosis of rejection is made after the advent of renal damage, it is not surprising that the necessarily late application of antirejection therapy often results in only partial restoration of renal-transplant function. Serial surveillance biopsies of the transplant, a maneuver that would undoubtedly detect some instances of subclinical rejection,[2] are precluded by cost and complication-related issues.

The advent of reverse transcriptase polymerase chain reaction and DNA-microarray technology has allowed for highly sensitive, accurate, and quantitative detection of the transcriptional profiles of tissue samples from recipients[3-12] and donors,[13] thereby enabling the discovery of a molecular signature for acute cellular rejection.[3-12] Acute allograft rejection is characterized by infiltration of the allograft by activated T cells. Accordingly, expression of T-cell–activation genes is evident in renal-transplant biopsy specimens obtained from patients who are undergoing transplant rejection.[2,4-12] Knowing that activated donor-specific cytotoxic T lymphocytes (CTLs) infiltrate rejecting allografts, the expression of T-cell–activation genes that control the cytolytic machinery of activated CTLs was first analyzed in renal-transplant biopsy specimens.[4-6]

Since the transplant is infiltrated by a T-cell–rich population of mononuclear leukocytes, robust intragraft expression of the T-cell–specific T-cell antigen receptor[7] and T-cell–specific CD3[8] genes are excellent markers for rejection. Nonetheless, infiltration with other mononuclear leukocytes is also noted during rejection. Particularly interesting is the observation that amplified expression

of the B-cell–specific CD20 gene in the context of robust expression of CTL-related genes and other activation genes provides a molecular signature for rejection episodes that are resistant to corticosteroid therapy.[9]

To enhance the use of transcriptional profiling methods, noninvasive techniques that do not rely on renal biopsy are desired. In fact, the molecular signature of rejection, amplified expression of CTL genes, can be detected in circulating blood cells at the time of rejection.[10] A drawback to the use of circulating blood is that blood cannot be used to analyze the heterogeneous population of mononuclear leukocytes that infiltrate rejecting allografts. In contrast, under most circumstances, lymphocytes that are present in the urine of patients with renal transplants have traversed the kidney before entering the urine flow. A notable innovation of Suthanthiran and colleagues has been the clever use of urine-sediment cells for transcriptional profiling studies.[8] Parallel studies comparing renal-transplant biopsy specimens and urine-sediment cells reveal a similar sensitivity and specificity of gene expression for CTL-effector molecules, CD3, and other genes for the diagnosis of rejection.[8] In this issue of the *Journal*, the Suthanthiran group has extended these studies in the study by Muthukumar et al.,[3] which reaffirms that amplified expression of CD3 (a T-cell-lineage–specific transcript), perforin (an activated CTL transcript), and CD25 (a T-cell–activation transcript) is far more robustly expressed in urine-sediment cells from renal-transplant recipients with acute rejection than in cells from patients with either chronic rejection or a normal biopsy.[3]

In addition, Muthukumar et al. have carefully studied *FOXP3* gene expression. Expression of the *FOXP3* gene, a member of the forkhead family of cell-differentiation genes, is a lineage-specific transcript for graft-protecting regulatory T cells.[14] *FOXP3* is the master switch that turns on the immunosuppressive properties of regulatory T cells.[14] The finding that increased *FOXP3* expression is a correlate of rejection is somewhat of a surprise. The molecular footprints for both cytopathic and protective cells are detected in T cells that have traversed the kidney and are collected from the urine sediment.[3] Thus, the complex nature of immune response to the kidney transplant at the cellular level is made evident by the lineage-specific gene expression detected within urinary-sediment cells. Rejection is orchestrated by cytopathic, tissue-injuring CD4+ helper type 1 and CD8+ CTL T cells, but the study by Muthukumar et al. demonstrates that some *FOXP3*-positive protective T cells are also present within the graft during rejection.

The revelation in the new study is the remarkable ability of *FOXP3* transcript levels to predict clinical outcomes. Although the molecular signature of rejection is present, low expression of *FOXP3* at the time of rejection forewarns that rejection is severe and may not readily respond to antirejection therapy. Moreover, low expression of *FOXP3* identifies transplants at heightened risk of graft failure within six months. Higher *FOXP3* transcript levels at the time of rejection, despite the molecular signature of rejection, heralds a more favorable clinical outcome. It is notable that the histologic grade of rejection (Banff score) does not predict the severity or clinical outcome of treated rejection episodes. Why? Pathological examination of a transplant biopsy can measure the magnitude and scope of graft infiltration by leukocytes, but routine pathological analysis is not informative as to the cellular program (destructive or protective) of the graft-infiltrating cells.

In animal studies, therapeutic regimens that successfully induce donor-specific tolerance and thereby allow the safe withdrawal of immunosuppressive therapy serve to tip the balance of immunity from the predominant cytopathic-type antidonor immunity detected in untreated hosts toward the enduring ascendancy of protective-type immunity.[15] The clinical data arising from the report of Muthukumar et al., taken together with experimental data, strengthen and add texture to the concept that the balance of cytopathic-type immunity to protective-type immunity determines both gross and subtle clinical outcomes, even in hosts receiving daily immunosuppressive therapy.

The potential for molecular diagnostic techniques to predict renal-transplant rejection, the safety of drug withdrawal, and other long-term and short-term outcomes may be substantial. The influence of assessing the molecular status of renal transplants in the operating room on later clinical outcome is also being analyzed.[13] Overall, molecular diagnostic strategies are being tested in multicenter clinical trials sponsored by the National Institutes of Health and the Immune Tolerance Network. If the validity of these methods can be confirmed, the door to more effective

and individualized therapy will be wide open. The present definition of "successful" treatment of a rejection episode is a loss of renal function of less than 15 percent. It would be a great improvement if the techniques described by Muthukumar et al. could lead to preemptive anticipation of problems and fully successful therapy.

Dr. Strom reports serving as a member of the Immune Tolerance Network.

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CLINICAL TRIAL REGISTRATION

The *Journal* encourages investigators to register their clinical trials in a public trials registry. The members of the International Committee of Medical Journal Editors plan to consider clinical trials for publication only if they have been registered (see N Engl J Med 2004;351:1250-1). The National Library of Medicine's www.clinicaltrials.gov is a free registry, open to all investigators, that meets the committee's requirements.